(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 February 2001 (01.02.2001)

PCT

(10) International Publication Number
WO 01/07079 A1

(51) International Patent Classification[7]: A61K 39/00, 39/08

(21) International Application Number: PCT/NZ00/00138

(22) International Filing Date: 26 July 2000 (26.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
334141 26 July 1999 (26.07.1999) NZ

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/07079 A1

(54) Title: IMPROVEMENTS IN DISPENSING VACCINES

(57) Abstract: The present invention is directed to a vaccine formulation providing for the extended release of antigenic material over time. The release profile of different embodiments can be varied, allowing a single administration to establish an active immunity in an animal, substantially equivalent to separately administered sensitising and booster shots according to conventional techniques. Preferred embodiments are solid or fluid based, and biodegradable. Variations allow for the periodic and/or sequential release of different antigenic material. Vaccines addressing members of the genus clostridium and their toxoids are specifically discussed.

IMPROVEMENTS IN DISPENSING VACCINES

TECHNICAL FIELD

This invention relates to improvements in the dispensing of vaccines.

More specifically, the present invention is directed to inducing an extended immune response in an animal over a period of time through the prolonged introduction of immunological material to a subject's system over an extended period of time. Particular reference is made to vaccines addressing clostridial diseases.

BACKGROUND ART

Vaccine technology has been used for many years to anticipate, control and prevent bacterial disease.

Immunity occurs naturally in animals as a consequence of an animal's exposure to foreign material in the environment. It is optimally developed when it results from the continuous ingestion of bacteria, which may be through the air, food, soil and other sources.

Immunity may be artificially induced by vaccination, which may be passive or active. Passive immunity is typically effected by the transfer of immunity from an immunised subject to a non-immune host by administering serum antibodies, or transplanting lymphocytes. Usually, however, a subject receiving a passive immunity acquires only a short-term protection.

In contrast, active immunity is based on the defensive reaction of the immune system of an animal to vaccination. Conventionally, the reaction develops 10-14 days after the first injection, and 1-3 days after an optional second or booster injection. Such booster injections are given to further stimulate active immunity. It is known that an animal responding to active immunisation produces a higher level of protection, which lasts longer and is very easy to boost upon subsequent vaccination.

The present invention has been developed particularly with the problems of clostridium bacteria in mind. In many clostridium bacteria (the genus belonging to the family (*Bacillacae*) have been identified as pathogenic or potentially pathogenic. These

bacteria often occur in water, soil, and in the intestinal tract of humans and animals. Total eradication of clostridial bacteria, and associated clostridial disease, is extremely difficult due to both their widespread presence and as these bacteria can exist for decades in soil in spore form.

Clostridium only produce disease when favourable conditions within sheep trigger off the growth and multiplication of the bacteria. Rather than the bacteria itself causing pathogenicity in most cases, it is in fact toxin produced by the bacteria which most seriously affects a subject. For a large and expanding bacterial population, toxin production can be significant and rapid. Often the poison or toxin produced by the multiplying bacteria is released so quickly that usually the first sign of clostridial disease is a dead animal. Accordingly treatment is rarely an option except where death in a flock or herd indicates that remaining members may be succumbing to clostridial bacteria.

The most effective control against clostridial disease is vaccination using the correct vaccine at the right time. However, correct timing for maximum vaccine effect can be difficult. Even where active vaccination techniques are used, these may be insufficient to fully protect animals from season to season. Flocks and herds of animals can be large making it expensive and laborious to preventatively administer booster vaccinations each season. This can also be wasteful in seasons where local, geographic, and climatic conditions may not favour the rapid growth of clostridial bacteria.

To date, vaccination techniques, in general, as well as those directed specifically to addressing clostridia, have followed the standard technique (for active vaccination) of administering a first primary sensitising vaccination followed by subsequent booster vaccinations. In practice however, most farmers will administer a primary sensitising vaccine but neglect to follow this up with a booster. Typically this is due to the time taken to re-gather a flock or herd and subsequently re-administer a booster vaccine. Without this booster, full immunity is not obtained and only partial protection is acquired. The duration of any acquired immunity is also diminished and it is likely that little residual protection will be retained for the next season.

It is also possible that larger doses may be administered in the hope of avoiding or reducing the need for subsequent booster shots though it is believed that large doses could possibly result in a growth penalty for the animal where the animal is young. This

may have financial consequences for a farmer at a later stage. Further, some animals become sick for a time after receiving a large dose of vaccine, and may need to be treated or cared further by a farmer which may be time consuming and expensive.

New Zealand Patent No. 286242, to CSL Limited and Ors, relates to an implant for veterinary or human application, which is configured to administer the release of an antigen at different times. The release at each specified interval occur as one or more (short delayed) pulses over a period of time after initial administration and relies primarily on coating technology to control each release period, though with no release of antigen between each pulse. It is considered that potentially there may be some advantage in continuously releasing a low level of antigen between each burst or pulse.

Further, current methods of delivery in the prior art relies largely on polymeric coatings and structures, which are non-dissolving and remain in the animal after the vaccine has been exhausted.

It is an object of the present invention to address the foregoing problems or at least to provide the public with a useful alternative, ideally providing a means of vaccination enabling a single administration to fulfil the requirements of traditional booster after sensitising vaccination.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

DISCLOSURE OF INVENTION

According to one aspect of the present invention there is provided a vaccine for the prolonged release of an antigenic substance for eliciting an immune response in a subject, said vaccine including at least one antigenic substance dispersed in a pharmacologically acceptable carrier system and characterised in that the antigenic substance will be released from the carrier system over a period of time after introduction of the vaccine into a subject.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which there is present antigenic material eliciting an immune response to bacteria of the genus *Clostridium*.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which there is present antigenic material for eliciting an immune response to any one or more of the group comprising: *C. tetani, C. perfringens, C. botulinum, C. novyi,* and *C. septicum.*

According to another aspect of the present invention there is provided a vaccine substantially as described above in which a quantity of antigenic substance is released substantially continuously, once release is initiated, for the intended life of the vaccine.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which there is provided an initial boosted release rate of antigenic substance.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which there is present a second boosted release rate of antigenic substance at a time interval after the initial boosted release.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the interval between the initial and second boosted release rates corresponds to a predetermined ideal period between sensitising and booster shots for conventional administration by injection of antigenic material to confer active immunity to a subject.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the time interval, measured between peak boosted release points, is within the range of 3 through 180 days inclusive.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the time interval, measured between peak boosted release points, is within the range of 5 through 35 days inclusive.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which there are present additional periods of boosted antigen release after intervals.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which, other than during specified periods of boosted

release, the release rate is substantially constant for the intended life of the vaccine, other than possible tailing off near the end of the life of the vaccine.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which antigen release occurs substantially immediately after introduction of the vaccine into the subject.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which substantially immediately after introduction is within 24 hours of introduction.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which initial antigen release is delayed.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the delay exceeds 24 hours.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the delay exceeds 5 days.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the delay is accomplished by any one or more of:

- encapsulating an antigen with carrier portion within a dissolvable or degradable protective layer;
- utilising a dissolvable or degradable cap portion to limit access to an antigen with carrier portion;

According to another aspect of the present invention there is provided a vaccine substantially as described above in which a boosted release rate of antigenic material is accomplished by providing a secondary portion of antigenic material within a carrier system and which secondary portion either or both of:

- has different release rate characteristics than the main antigen with carrier portion, and
- has an exposed surface area in addition to the main antigen with carrier portion.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which a boosted release rate of antigenic material are accomplished by providing supplementary portions of antigenic material within a carrier system, and which supplementary portion either or both of:

- has different release rate characteristics than the main antigen with carrier portion, and
- has an exposed surface area in addition to the main antigen with carrier portion.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which during said periods of boosted release of antigenic material, different antigenic substances to that of the main antigen with carrier portion are additionally or alternatively released.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which at least one of said different antigenic substances are directed to a member of the species clostridium or possesses an antigenic determinant therefor.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which an antigenic substance employed comprises inactivated or attenuated bacterial cells.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which an antigenic substance employed comprises material possessing an antigenic determinant capable of eliciting an immune response to one or more members of the genus *Clostridium*.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which an antigenic substance employed includes material possessing an antigenic determinant portion capable of eliciting an immune response to one or more toxins produced by a member of the genus *Clostridium*.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the carrier system in which an antigenic substance is present comprises a matrix through which said antigenic substance is

dispersed; the matrix slowly dissolving when exposed to the environment into which it is introduced.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the matrix is gel-like in consistency.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the matrix is substantially solid or includes solid portions.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the carrier system is fluid, and forms a matrix after introduction into the subject.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the carrier system includes or comprises a polycaprolactone material.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the carrier system comprises a hydrogel encapsulating antigenic material.

According to another aspect of the present invention there is provided a vaccine substantially as described above substantially in the form of a bolus for sub-dermal implantation.

According to another aspect of the present invention there is provided a vaccine substantially as described above which, in its entirety, is substantially biodegradable.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which the carrier system is biodegradable within the subject to which it is introduced.

According to another aspect of the present invention there is provided a vaccine substantially as described above in which any coating portions are biodegradable.

According to another aspect of the present invention there is provided a vaccine substantially as described above which includes a body or shell, and wherein said body or shell is biodegradable.

According to another aspect of the present invention there is provided a vaccine substantially as described above whose active lifetime is within the inclusive range of 5 through 360 days.

According to another aspect of the present invention there is provided a vaccine substantially as described above whose active lifetime is within the inclusive range of 7 through 60 days.

According to a further aspect of the present invention there is provided a method for vaccination comprising the introduction of a vaccine as claimed in any one of the preceding claims into an animal other than a human.

According to another aspect of the present invention there is provided a method, substantially as described above, in which the vaccine is directed to either or both of members of the genus clostridium, and toxins produced by said members.

According to a further aspect of the present invention there is provided a method for conferring active immunity comprising the introduction, into an animal other than a human, of a substantially as described above which is configured to actively release antigenic material into the subject over a period exceeding 7 days, and wherein when the expected release profile of the antigenic material over time is viewed, there are present at least two periods of boosted release of antigenic material, and wherein both periods of boosted release substantially peak within 60 days of initial release of antigenic material from the vaccine.

According to another aspect of the present invention there is provided a method, substantially as described above, in which the vaccine is directed to either or both of members of the genus clostridium, and toxins produced by said members.

According to another aspect of the present invention there is provided a method, substantially as described above, in which the vaccine is introduced into a subject by injection.

According to another aspect of the present invention there is provided a method, substantially as described above, in which the vaccine gels or solidifies once introduced into the subject.

According to a further aspect of the present invention there is provided a vaccine when introduced into an animal as a means of vaccination.

According to a further aspect of the present invention there is provided the production of an immune response in an animal through the introduction of a vaccine, substantially as described above.

The present invention will generally take the form of a vaccine which, once introduced into a subject's system can induce an immune response in a subject over a period of time. Typically the vaccine will be tailored for parenteral delivery, and may be an implantable device, often introduced by sub-dermal implantation. This is not to preclude the non-parenteral delivery of embodiments of the present invention, though in most instance sub-dermal placement is preferred.

Preferably the vaccine will contain one or more antigenic substances capable of eliciting an immune response in the subject. Embodiments of the present invention are often generally characterised that once activated, there will be a continuous release of antigenic material throughout the lifetime of the device. This is in contrast to prior art such as the CSL device which releases doses of 'vaccine' or other materials at intervals with lulls (during which there is no release) in between.

While the rate and amount of release of antigenic material may be substantially constant throughout the life of the device, this need not be so in all embodiments. Similarly, while the rate of continuous release will often be relatively low, again this may vary among embodiments. By way of example, some variations will be described. The first is a simple arrangement in which antigenic material is released substantially constantly throughout the life of the implant. There may be some tailing off as the implant reaches the end of its lifetime. Such embodiments may find use as boosters, administered in a season following initial vaccination (establishing active immunity) to maintain or re-activate immunological activity. In such cases the rate of release may be relatively short (e.g. over a period typically of 1 month or less) and/or at low levels to avoid de-sensitisation occurring instead.

In another variation, the above constant release profile may be modified through having a boosted initial release rate. This will subject the animal to a higher initial presence of antigens to effect a rapid and more intense immune response. This is followed by a more steady and slower release of antigenic material over a prolonged period of time so the level of immunity may be reinforced within the animal, though again avoiding de-sensitisation problems.

Another variation is to periodically supplement a relatively constant rate with burst of higher release. This may be used to trigger the immune system to remain highly active over time. This is the preferred arrangement for a vaccine product, with a first, primary sensitising boosted release of antigenic material followed by a secondary boosted release a period of time thereafter, typically taking the place of the booster shot in conventional vaccination techniques. There may be a low release rate of antigenic material between the boosted releases, and optionally after the last boosted release rate as well.

Other variations are possible. In each case, the constant release profile need not necessarily be a substantially flat and level plateau (other than boosted release portions), but may also resemble a decay curve. However, it is desirable that this decay curve still releases sufficient amount of antigens to stimulate a useful level of immune response.

As can be appreciated, it is possible that several smaller administrations of vaccine may be introduced into an animal rather than one larger vaccination. For solid embodiments this may mean the implantation of several smaller devices, and each may vary. For instance, each may have different release profiles so that the desired overall release profile may be attained. These techniques are also applicable for liquid compositions, including those embodiments which may harden in situ. Such options are largely a matter of user choice, though will be influenced by factors such as what represents a convenient size implant or injection for a particular size of animal, as well as the size, type, and susceptibility of the animal, geographic and climatic considerations etc. Such multiple vaccinations may be introduced sequentially – e.g. with a single injection – to reduce labour for the farmer or veterinarian.

In terms of the lifetime of the implant, which may be considered to be the period throughout which useful amounts of antigenic material are released (to result in or maintain a useful level of immune activity) it is preferable that the lifetime of the implant is at least seven days, though more preferably in excess of one month. Such

shorter lifespan devices will still represent a useful alternative to traditional primary and booster injections, as well as potentially eliciting a higher degree of immune response due to constant exposure of the animal to antigens over a prolonged, even though still relatively short, period of time.

A number of embodiments will have a lifetime exceeding three months, and even more preferably, will have a lifetime in excess of one year though typically (but not necessarily) less than 20 months. However, for these longer duration devices, the release rate over time (after initial release periods) may be relatively low or negligible, though are likely to present a further boosted release portion some considerable time interval after initial release or activation. By way of example, such an embodiment may present an initial sensitising boosted release period, followed by a 'booster' boosted release period a relatively short period thereafter – for instance inside 3 months. After a further period, say around 9-15 months from the initial release period, a further 'booster' boosted release period may occur. Different antigens may also be released at different periods of time as well. It is also possible that more periodically a higher release rate may be released to act as a 'booster' though care should be taken so that the release amount and periodicity does not in fact result in desensitising of the subject to released antigenic material.

The antigenic substances included in the implant may vary. Most preferably these will be substances and materials able to elicit an immune response to either or both of bacteria of the genus *Clostridium*, and/or toxins produced by said bacteria. The choice of antigenic materials may vary. These may represent attenuated or inactivated bacterial material and/or reproductive material. They may also include other antigenic material possessing a determinant able to produce the desired immune response.

Another variation is to provide an implantable device eliciting a passive type immune response, and including purified antisera and/or other passive immunological material. However, more preferably the use of such passive immunological substances will be to supplement or complement antigenic material eliciting an active immune response. It is also possible in such embodiments that such passive immunological agents are released during the initial period of the lifetime of the device. This may be especially useful where the implantable device is administered to animals suspected of already being affected by clostridia, and where agents able to address possible toxin poisoning, or able

to assist the subject in dealing with such toxins, are included. As can be appreciated there are a number of possible variations and combinations to produce embodiments which include both active and passive immunological materials. All the possible permutations and combinations are too numerous to mention and it is assumed that a skilled addressee of the art, having been alerted to the possibilities, will appreciate that a wide range of possibilities exist and fall within the scope of the present invention.

Methods of construction of devices according to the present invention may vary. To some extent construction will be determined by the desired release profile.

In most embodiments the active material (e.g. the antigenic substance and/or passive immunological substances) will be encapsulated or contained in slow release matrix. Virtually any system which allows the slow release of active substance over time once implanted in its final environment, will be suitable providing that it is pharmacologically acceptable. However, preferred embodiments of the present invention disperse or encapsulate the active substance in what will be conveniently described as a carrier system. This carrier system will preferably comprise a slowly dissolvable solid or gel matrix, and may make use of hydrogel technology which can be used for the slow release of materials into a 'wet' or internal environment. Many types of solid matrix are known and used for slow release boluses and the like, and may be employed in the present invention.

A simple embodiment of the present invention will comprise essentially a bolus of substantially solid construction and which, once implanted, may slowly dissolve about its entire outer surface area. As the size of the bolus decreases throughout its life, then the release rate of active material will also decline as well. In most instances this will be of little consequence, and it will be appreciated that the shape of the solid device can be chosen to affect both initial release rates and the effect of any tailing off thereof.

A further variation of the above type of construction is to have multiple layers, or graduated layers. This can allow for differing release rate profiles merely by adding layers of different solubility and/or release rates, or containing different concentrations of active material. For instance there may be a core layer allowing for a relatively high release rate – this will represent a boosted release rate which acts as a booster for the animal. Surrounding this may be a further dissolvable layer though with only a low release of active material, so that antigen release remains relatively low and primarily to

keep the immune system stimulated. Surrounding this will be another layer with a relatively high release rate, which represents the boosted sensitising release period. About this may be a protective coating (for handling) and which may also delay initial activation of the implant. As can be appreciated, this layer technology can be built up to allow for more complex release profiles, and to also be used to allow for the release of different active substances as well. This may be used to extend the versatility of the device to also, at certain times, release active substances which may address not only clostridia and/or their toxins, but also other substances and organisms for which it is desirable to confer immunity. It is also noted that in such embodiments, rather than having inactive layers introducing delays between each 'level', the layers representing delays between periods of 'boosted' response can still release antigenic material. This may be at lower levels to provide for heightened immune stimulation, but avoiding desensitisation, or may allow different antigens (or antisera) to be alternately release according to user choice. Such embodiments are in contrast to prior art slow release devices.

As indicated, protective coatings may also be applied to such solid embodiments to protect them during handling. These outer protective coatings can also influence any initial delay between implantation and activity. Hence, activity may be relatively rapid (e.g. within 24 hours of implantation) or alternatively may delay any significant initial activity for a period of time – e.g. greater than 24 hours. Again, this will be influenced also by the physiology of the animal, and the implant site.

Another variation to a tableted or bolus type embodiment is to provide a shell or casing in which active material and its carrier are contained. This is more particularly suitable for but not restricted to carrier systems whose integrity may not be able to be maintained as a solid tablet. Such embodiments are particularly suitable for more fluid, and hydrogel type carrier systems. A further potential advantages of 'cased' embodiments is that the exposed surface area (and hence release rate) is generally dependent upon a provided aperture or opening whose size will typically remain constant throughout the life of the implant. Hence a more constant release rate is possible with such embodiments. However, the preference for cased embodiments in to use a biodegradable casing so that solid foreign matter does not contaminant the carcass of a slaughtered animal. Potentially, the use of biodegradable substances has advantages in that the implant will dissolve after the vaccine is exhausted. This potentially expands

the possible locations for insertion sites on the animal, as there may be less concern about having a foreign object in areas normally associated with consumption. The implant will potentially dissolve, leaving minimal or no waste. As will be mentioned below, casings may be simulated by suitable longer-lasting dissolvable coatings about 'firmer' implants though this technique would not be applicable to more fluid type vaccines.

Such 'cased' embodiments are also quite adaptable for allowing different release profiles to be employed. Altering the internal configuration and dimensions of the body shell or casing can be used to affect release rates over time. Furthermore, the body may be filled in layers which in many instances may be easier (from a manufacturing point of view) than employing coating techniques.

Body cases or shells with multiple chambers may also be used to achieve more complex release profiles. This enables active/carrier combinations of different types to be placed in different chambers. For instance, to allow for a high initial release rate, a small second chamber may be provided. Once activated, active material will be released from the smaller as well as the primary chambers resulting in a relatively high release rate. However, once the secondary chamber runs out, the release rate will revert to that normal for the main chamber.

As a further variation, seals of different characteristics may be used to seal each chamber. Some may be rapidly dissolving seals while others may take quite some period of time. This can be used to control the timing of release of different materials, and would be of particular suitability where occasional pulses of active material are to be released periodically to supplement the primary continuous release profile.

Another consideration is the material from which the casing or body is manufactured. Ideally this will be a biodegradable substance so that slowly over time it will dissolve or degrade. Ideally, this will approximate or exceed the anticipated lifetime of the implant, though casings which are expected to degrade or disintegrate before the anticipated lifetime of the bolus (though typically towards the end of that lifetime) may also be considered in certain cases. Use may be made of this degradation to alter or affect release rate profiles.

As a further variation, a solid implant may be coated over the majority of its surface with a slowly dissolving or degradable material to approximate a shell or casing. The resulting characteristics of such an embodiment will resemble that of a 'cased' embodiment, though the method of construction will vary (e.g. a coating selectively applied to an already formed solid core, as opposed to material filled into an already existing body).

Reference to an implant may be made with reference to a solid preparation of a size and shape suitable for parenteral implantation, and that releases the vaccine over an extended period of time. The aforementioned definition is consistent with the definition in "British Pharmacopia 1998 Volume 2."

In some embodiments, the pharmaceutically acceptable carrier system may be a hydrogel. Such materials are well known in the industry, and may typically include a wide range of swellable materials (when exposed to water or allowed to hydrate) including, for instance, varying modified celluloses, PLGA polymers, and various other polymers.

Other embodiments may represent a fluid vaccine which stiffen or solidify after introduction into the animal. A suitable carrier for such a vaccine are polycaprolactone polymers. Such embodiments can assist easy injection, and with reduced stress to the animal, yet represent an embodiment allowing for prolonged antigen release sufficient to create a useful active immunity in the animal.

As can be appreciated, it is not possible to effectively layer liquid vaccines to allow for varying release profiles. However, such fluid vaccines can be used in a number of ways. For instance, they may be used to provide a low and substantially constant release profile of antigenic material while other injected material provides for any boosted release periods. This other injected material may be solid or gel-like implants either injected at the same time, or substantially co-administered. This other injected material may also represent other fluid vaccines, co-administered or injected at the same time. For instance, a pre-prepared syringe may include a number of layers of fluid vaccine, each with differing release profiles and/or solubility, so that the accumulative release profile approximates the desired profile. At the time of injection, each of the layers are sequentially injected into the animal as part of a single injection. Hybrid vaccines comprising both solid and fluid components may also be introduced in such a manner as

part of a single injection.

The present invention may provide a number of potentially realisable advantages. The present invention takes advantage of potentially enhanced immunological response being developed by a continuous exposure to a vaccine over a period of time, and ideally supplementing boosted release periods, as contrasted to periodic release bursts without any release in between.

The present invention can reduce the labour intensity, and the cost associated with that labour intensity, as was previously necessary when administering the vaccines. One dose may be given, to provide an improved and optimal immunological response. The ability to stagger the release of different antigens can represent an advantage by allowing the subject's immune system to be exposed to a continuing series of different antigens (as an alternative to collective introduction of a number of antigens). This can be of use in addressing afflictions such as clostridial disease, where a number of different organisms can be responsible. A potentially realisable advantage of such staggered release is heightened or enhanced immunity to the specific organisms (and/or toxins). By staggering or alternating the release of different antigenic material, an immune system may also be kept in a heightened state of activity with reduced risk of desensitisation against one specific antigen. Hence, this is typically a preferred option for long-lived continuous release embodiments where the released antigenic material is gradually alternated over any extended release period.

A number of variations of the invention can be seen to exist. It is anticipated that this will allow the user a great deal of flexibility, particularly in addressing problems associated with clostridia. It is envisaged that a skilled addressee of the art will be able to implement the many possible embodiments and variations of the present invention given the description herein.

BRIEF DESCRIPTION OF DRAWINGS

Further aspects of the present invention will become apparent from the following description which is given by way of example only and with reference to the accompanying drawings in which:

Figure 1 shows a schematic representation of one embodiment of the present

invention in the form of a solid implant,

Figure 2 is a diagrammatic view of a preloaded syringe with fluid injectable vaccine representing another embodiment of the present invention, and

Figures 3a-d are a selection of possible release profiles according to varying embodiments of the present invention.

BEST MODES FOR CARRYING OUT THE INVENTION

Example 1

With reference to Figure 1 there is shown a possible configuration of a solid implantable device according to the present invention shown by general arrow 1.

The device 1 may be manufactured from a number of substances. A number of substantially insoluble calcium compounds are commonly used for slow release bolus construction and are suitable for tableting. This includes compounds such as calcium phosphate, which may be used as a matrix impregnated with active material.

Active material will typically include antigenic material capable of eliciting an active immune response to one or more members of the clostridia genus and/or toxins thereof. Antigenic material used may include inactivated cell material or fragments thereof, as well as other commonly used antigenic material including proteinaceous or other substrate materials with appended fragments including an antigenic determinant for the desired species and/or toxins. Toxin antigens may comprise modified chemical structures with appropriate antigenic determinants appended thereto, or modified toxins which have been structurally or chemically modified to no longer be toxic. For non-liquid embodiments of the invention, freeze-dried concentrates may be considered, including concentrates of clostridium toxoids and anacultures. Virtually any commercially available antigenic material, or technique for producing antigenic material, may be relied upon as a source of active material for use in the present embodiment.

The impregnated or combined matrix is typically tableted into a solid material such as illustrated as any of portions 4, 5, or 6 in figure 1. In that figure, there are shown 3 discrete portions, each representing a differing release rate characteristic. The

preference is for a high release portion at the ends (4 and 6) with a slower release portion 5 in the middle. Outer slowly dissolving coating 2 controls release so that the portions (4,5,6) dissolve sequentially from one end. A faster dissolving end coating 3 exposes the first end 4 so release may begin.

Differing release rates can be controlled by adjusting the dissolution rate of the matrix for each portion, using conventional techniques. This may include the use of differing materials, or faster dissolving (within the environment to which the implant is introduced) fillers within the matrix.

The multiple portion implant 1 can be tableted using conventional techniques, into the sequentially layered form of figure 1. The coatings can also be applied according to conventional technologies, and may use any material suitable for the task, though are preferably biodegradable. Materials which may be considered for use include various glycolic and lactic acid (PLGA) with ethylcellulose mixtures, varying polycaprolactone polymers, and slowly dissolving polymeric materials such polyvinyl alcohols etc. A wide range of other materials are also available.

The coating 2 may also represent a hard shell or casing which is filled and end sealed (and in such an alternative arrangement, the portions (4-6) may not necessarily be hard or solid matrices) though the shell or casing should be biodegradable over time.

Preferably the antigenic material will address a number of clostridial strains. The preference is to include antigenic material directed to all of the following species, though this may be varied according to user requirements:

(a) Clostridium tetani,
(b) Clostridium perfringens,
(c) Clostridium botulinum,
(d) Clostridium novyi,
(e) Clostridium septicum

As a variation, different antigens or mixtures of antigens may be provided in each layer. For instance, different antigenic material may be released in middle portion 5. This may also be in a boosted release rate, and may represent the initial sensitising dose for the selected antigens. A further layer adjacent portion 6 may also be provided to represent

the 'booster' release of the antigens of portion 5 after portion 6 has dissolved.

Example 2

In this example, the antigenic material (such as discussed in example 1) is dispersed throughout a poly-ortho ester polymeric material. The polycaprolactone is selected to be fluid for the purpose of injection, but to stiffen or harden into a substantially non-fluid depot after injection into the subject. Multiple or varied release profiles may be achieved by coadministering or sequentially administering differing formulations — which either or both have differing dissolution rates, or included antigenic material doses. Figure 2 illustrates a pre-prepared syringe (10) with multiple layers (11-14) which are sequentially injected. This effectively represents an injectable fluid equivalent to the multiple solid portions of example 1.

Figure 3 represents some possible variations of release profiles. In these illustrative graphs (by way of example only), the characters (A-C) are used to represent varying antigenic formulations, with X(s) representing a primary sensitising release and X(b) representing a subsequent booster release (in each case a boosted release of material). These may be antigenic material for different species, combinations of antigenic material for a number of different species, antigenic material for one or more toxins, antisera, passive immunogenic material (for a passive immunity), and/or a combination thereof. These variations are purely to illustrate some of the possible options which may be implemented with various embodiments of the present invention.

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto within the spirit of the invention and as defined also in the appended claims.

It should also be appreciated that the term 'comprise' as used herein is not intended to be used in a limiting sense, and allows for the optional inclusion of other components or method steps (unless otherwise specified) in addition to those listed.

THE CLAIMS DEFINING THE INVENTION ARE:

1. A vaccine for the prolonged release of an antigenic substance for eliciting an immune response in a subject, said vaccine including at least one antigenic substance dispersed in a pharmacologically acceptable carrier system and characterised in that the antigenic substance will be released from the carrier system over a period of time after introduction of the vaccine into a subject.

2. A vaccine as claimed in claim 1 in which there is present antigenic material eliciting an immune response to bacteria of the genus *Clostridium*.

3. A vaccine as claimed in claim 2 in which there is present antigenic material for eliciting an immune response to any one or more of the group comprising: *C. tetani, C. perfringens, C. botulinum, C. novyi,* and *C. septicum*.

4. A vaccine as claimed in any one of the preceding claims in which a quantity of antigenic substance is released substantially continuously, once release is initiated, for the intended life of the vaccine.

5. A vaccine as claimed in any one of the preceding claims in which there is provided an initial boosted release rate of antigenic substance.

6. A vaccine as claimed in claim 5 in which there is present a second boosted release rate of antigenic substance at a time interval after the initial boosted release.

7. A vaccine as claimed in claim 6 in which the interval between the initial and second boosted release rates corresponds to a predetermined ideal period between sensitising and booster shots for conventional administration by injection of antigenic material to confer active immunity to a subject.

8. A vaccine as claimed in claim 6 in which the time interval, measured between peak boosted release points, is within the range of 3 through 180 days inclusive.

9. A vaccine as claimed in claim 6 in which the time interval, measured between peak boosted release points, is within the range of 5 through 35 days inclusive.

10. A vaccine as claimed in any one of claims 6 through 9 in which there are present additional periods of boosted antigen release after intervals.

11. A vaccine as claimed in any one of the preceding claims in which, other than during specified periods of boosted release, the release rate is substantially constant for the intended life of the vaccine, other than possible tailing off near the end of the life of the vaccine.

12. A vaccine as claimed in any one of the preceding claims in which antigen release occurs substantially immediately after introduction of the vaccine into the subject.

13. A vaccine as claimed in claim 12 in which substantially immediately after introduction is within 24 hours of introduction.

14. A vaccine as claimed in any one of claims 1 through 6 in which initial antigen release is delayed.

15. A vaccine as claimed in claim 14 in which the delay exceeds 24 hours.

16. A vaccine as claimed in claim 14 in which the delay exceeds 5 days.

17. A vaccine as claimed in claim 14 in which the delay is accomplished by any one or more of:

- encapsulating an antigen with carrier portion within a dissolvable or degradable protective layer;
- utilising a dissolvable or degradable cap portion to limit access to an antigen with carrier portion;

18. A vaccine as claimed in any one of claims 5 through 10 in which a boosted release rate of antigenic material is accomplished by providing a secondary portion of antigenic material within a carrier system and which secondary portion either or both of:

- has different release rate characteristics than the main antigen with carrier portion, and
- has an exposed surface area in addition to the main antigen with carrier portion.

19. A vaccine as claimed in any one of claims 5 through 10 in which a boosted release rate of antigenic material are accomplished by providing supplementary portions of antigenic material within a carrier system, and which supplementary portion either or both of:
    - has different release rate characteristics than the main antigen with carrier portion, and
    - has an exposed surface area in addition to the main antigen with carrier portion.

20. A vaccine as claimed in either claim 18 or claim 19 in which during said periods of boosted release of antigenic material, different antigenic substances to that of the main antigen with carrier portion are additionally or alternatively released.

21. A vaccine as claimed in claim 20 in which at least one of said different antigenic substances are directed to a member of the species clostridium or possesses an antigenic determinant therefor.

22. A vaccine as claimed in any one of the preceding claims in which an antigenic substance employed comprises inactivated or attenuated bacterial cells.

23. A vaccine as claimed in any one of the preceding claims in which an antigenic substance employed comprises material possessing an antigenic determinant capable of eliciting an immune response to one or more members of the genus *Clostridium*.

24. A vaccine as claimed in any one of the preceding claims in which an antigenic substance employed includes material possessing an antigenic determinant portion capable of eliciting an immune response to one or more toxins produced by a member of the genus *Clostridium*.

25. A vaccine as claimed in any one of the preceding claims in which the carrier system in which an antigenic substance is present comprises a matrix through which said antigenic substance is dispersed; the matrix slowly dissolving when exposed to the environment into which it is introduced.

26. A vaccine as claimed in claim 25 in which the matrix is gel-like in consistency.

27. A vaccine as claimed in claim 25 in which the matrix is substantially solid or includes solid portions.

28. A vaccine as claimed in either claim 26 or claim 27 in which the carrier system is fluid, and forms a matrix after introduction into the subject.

29. A vaccine as claimed in claim 28 in which the carrier system includes or comprises a polycaprolactone material.

30. A vaccine as claimed in any one of claims 1 through 24 in which the carrier system comprises a hydrogel encapsulating antigenic material.

31. A vaccine as claimed in either of claims 25 or 27 substantially in the form of a bolus for sub-dermal implantation.

32. A vaccine as claimed in any one of the preceding claims which, in its entirety, is substantially biodegradable.

33. A vaccine as claimed in claim 32 in which the carrier system is biodegradable within the subject to which it is introduced.

34. A vaccine as claimed in claim 17 in which any coating portions are biodegradable.

35. A vaccine as claimed in any one of the preceding claims which includes a body or shell, and wherein said body or shell is biodegradable.

36. A vaccine as claimed in any one of the preceding claims whose active lifetime is within the inclusive range of 5 through 360 days.

37. A vaccine as claimed in any one claims 1 through 35 whose active lifetime is within the inclusive range of 7 through 60 days.

38. A method for vaccination comprising the introduction of a vaccine as claimed in any one of the preceding claims into an animal other than a human.

39. A method as claimed in claim 38 in which the vaccine is directed to either or both of members of the genus clostridium, and toxins produced by said members.

40. A method for conferring active immunity comprising the introduction, into an animal other than a human, of a vaccine as claimed in any one of claims 1 through 37 which is configured to actively release antigenic material into the subject over a period exceeding 7 days, and wherein when the expected release profile of the antigenic material over time is viewed, there are present at least two periods of boosted release of antigenic material, and wherein both periods of boosted release substantially peak within 60 days of initial release of antigenic material from the vaccine.

41. A method as claimed in claim 40 in which the vaccine is directed to either or both of members of the genus clostridium, and toxins produced by said members.

42. A method as claimed in either claim 40 or 41 in which the vaccine is introduced into a subject by injection.

43. A method as claimed in claim 42 in which the vaccine gels or solidifies once introduced into the subject.

44. A vaccine as claimed in any one of claims 1 through 37 when introduced into an animal as a means of vaccination.

45. The production of an immune response in an animal through the introduction of a vaccine as claimed in any one of claims 1 through 37.

46. A vaccine, substantially as described herein, with reference to the accompanying drawings and contained examples.

47. A method of vaccination utilising a vaccine, said method being substantially as described herein with reference to the accompanying drawings and contained examples.

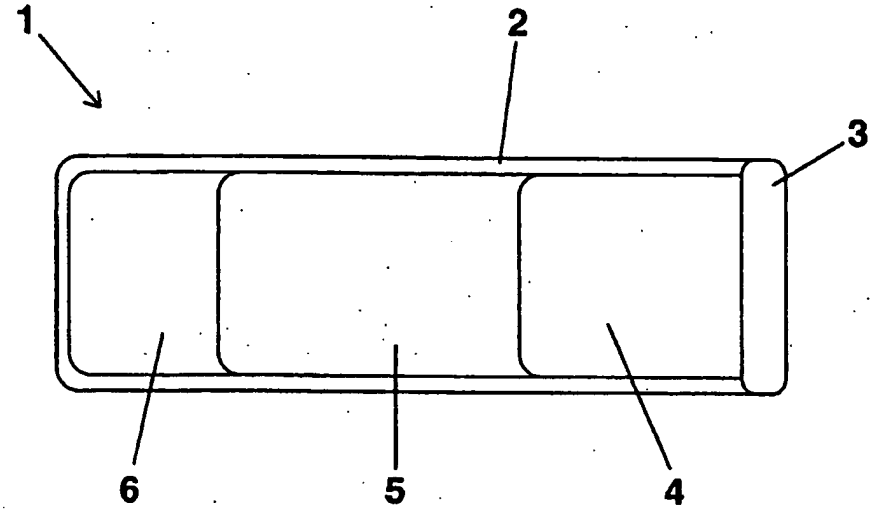


Figure 1

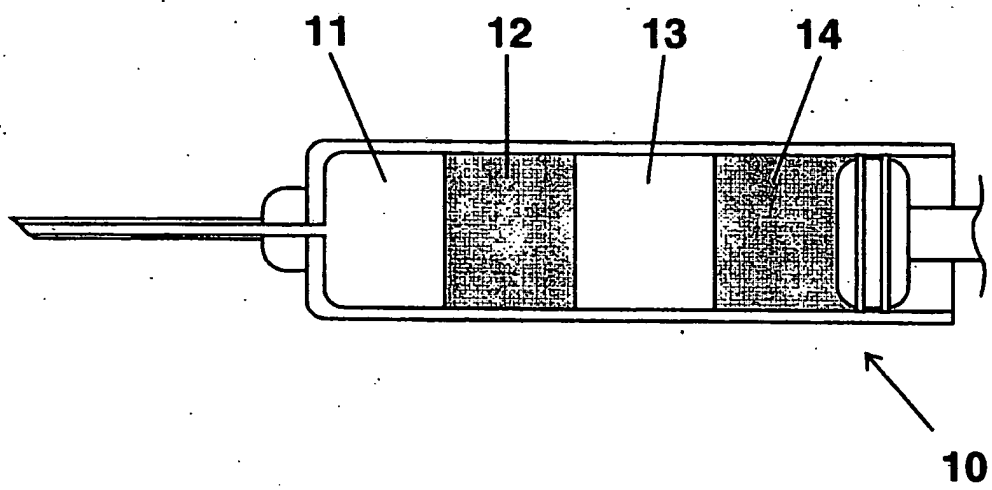


Figure 2

Figure 3a

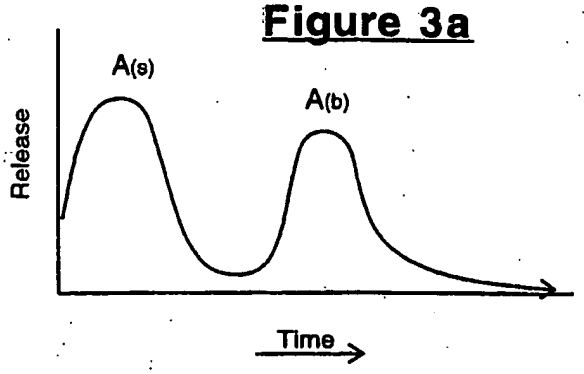


Figure 3b

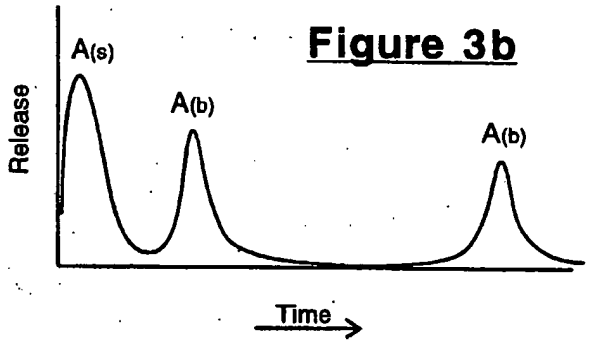


Figure 3c

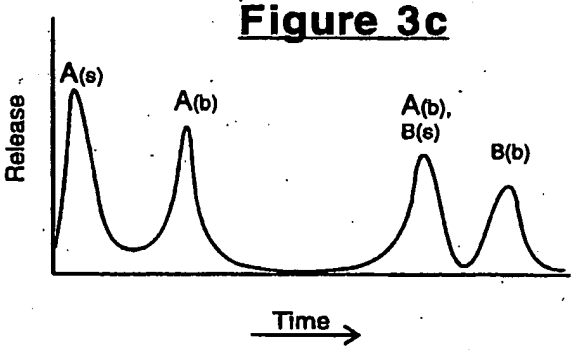
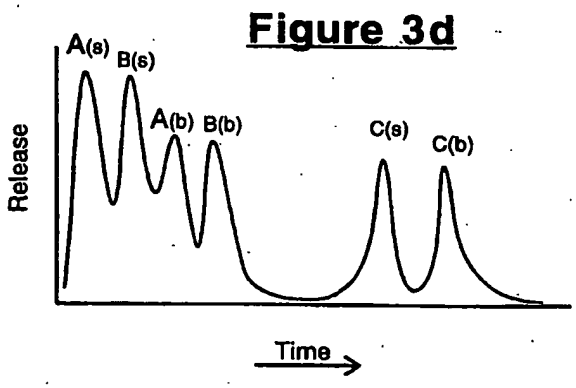


Figure 3d



INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00138

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 39/00, 39/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEE DATABASES BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, MEDLINE: Keywords used - clostrid?, tetanus, vaccine, antigen, delayed release, extended release, prolonged release, slow release, implant, carrier, matrix

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 626 170 A2 (SANDOZ LTD. et al.) 30 November 1994. See entire document, in particular examples 3 and 4.	1-47
X	PHARMACEUTICAL RESEARCH, volume 15, number 7, pages 1103-1110 (1998) by Johansen P. et al. "Improving stability and release kinetics of microencapsulated tetanus toxoid by co-encapsulation of additives". See entire document.	1-47
X	PHARMACEUTICAL RESEARCH, volume 15, number 7, pages 1111-1116 (1998) by Audran R. et al. "Enhanced immunogenicity of microencapsulated tetanus toxoid with stabilizing agents". See entire document.	1-47

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
14 December 2000

Date of mailing of the international search report
20 DEC 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00138

C.(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DEVELOPMENTS IN BIOLOGICAL STANDARDIZATION, volume 92, pages 63-78 (1998) by Gupta R.K. et al. "Biodegradable polymer microspheres as vaccine adjuvants and delivery systems". See entire document.	1-47
X	VACCINE, volume 14, number 15, pages 1442-1450 (1996) by Men Y. et al. "Induction of sustained and elevated immune responses to weakly immunogenic synthetic malarial peptides by encapsulation in biodegradable polymer microspheres". See entire document.	1-47
X	PHARMACEUTICAL SCIENCES, volume 85, number 6, pages 547-552 (1996) by Sanchez A. et al. "Pulsed controlled-release system for potential use in vaccine delivery". See entire document.	1-47
X	VACCINE, volume 11, issue 5, pages 596-597 (1993) by Aguado M. T. "Future approaches to vaccine development: Single-dose vaccines using controlled-release delivery systems". See entire document.	1-47

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/NZ00/00138

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
EP	0626170	CA	2123144	GB	9309543
		JP	6340543	JP	11193300

END OF ANNEX